Rapid Authentication of Andean Flours via Infrared Spectrometry and Gas Chromatography

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By

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Abstract

In recent years consumers in the United States have become more health conscious and have placed much more importance on a food's nutritional benefits. Andean grain flours exhibit many of the desired nutritional characteristics, such as high protein content, presence of essential amino acids, good source of dietary fiber, and being rich in the "heart healthy" Omega-3 and Omega-6 fatty acids. Due to the increased demand for these healthy grain alternatives there is risk of adulteration with less-expensive grains. The experimental goal was to develop a non-destructive analytical method that could swiftly categorize Andean flours based on flour type and separate pure grain flours from adulterated mixes. Pure Andean flours and ingredients were provided by Universidad Nacional Agraria la Molina (UNALM) (Lima, Peru). Whereas, commercial samples of the grain flours were attained from various local markets (Lima, Peru). Spectral data was collected from the Andean grain flours using a portable attenuated total reflectance (ATR) mid-infrared spectrometer equipped with a diamond crystal. Fat was extracted from the grain samples, analyzed with gas chromatography, and the data was used to create fatty acid profiles. The results show that samples could be separated by flour type based on spectral data and fatty acid profiles. The fatty acid profile showed differences in composition of major fatty acids (C16:0, C18:0, C18:1 and C18:2) present in flours, helping to identify potential adulterated samples. Additionally, the data shows that there was some prevalence of adulteration in market samples. Overall, a rapid analytical method was found that can be used "in-field" and provides the accurate recognition of adulterated food ingredients, making it a great alternative to conventional testing methods.

Introduction

Today, the Andean region is world renowned as a center for unique and diverse grain production. Canihua, Kiwicha (Amaranth), Maca, and Quinoa are indigenous food plants found in the Andean region that were first utilized thousands of years ago. Many years ago the Incas discovered increased nutritional value presented by these food plants, and began milling these crops. Milling made it possible for those living in rural areas to benefit from the nutrients found in the food plants, which compensated for the lack of animals present in the countryside that could be used as protein sources (Jaskoski 2013). Andean grains have the ability to function as bioactive ingredients in food products due to their increased levels of dietary fiber and natural antioxidants, such as phenolic compounds (Valcarcel-Yamani & Caetano da Silva Lannes 2012).

Andean grains have agronomic importance worldwide because they possess the ability to adapt in dissimilar environments. For example, the humidity and temperature requirements for Quinoa's growth are widespread and can be fulfilled by different ecotypes. This allows Quinoa to be grown in Europe, North America, Asia, Africa and Australia (Jacobsen 2003). Quinoa was selected by FAO as one of the crops to offer food security in the current century due to its increased consumer demand (FAO 2012). Therefore, due to the increased demand for these healthy grain alternatives there is risk of adulteration with less-expensive grains (Rossell 2013). Infrared spectroscopy combined with pattern recognition analysis is an attractive technology for fast, sensitive, and high-throughput detection of adulteration and contaminants in foods because of its fingerprinting characteristics (Rodriguez-Saona and Allendorf, 2011). Overall, the objective of this study was to develop a rapid testing method that combines data attained from IR spectroscopy to detect possible adulteration in native Andean flours. Fatty acids profiles were used to help characterize Andean flours for possible adulteration.

Materials and Methods

Flour samples were provided by the Universidad Nacional Agraria – La Molina (UNALM) located in Peru. UNALM was able to confirm that the samples provided were comprised of pure grain and had not been adulterated. Additionally, flour samples commonly sold in various Peruvian markets were also provided for comparison to the pure flour samples. The flour samples supplied are: canigua, cebada (barley), cebada tostada (toasted barley), haba (fava bean), haba tostada (toasted fava bean), kiwicha (amaranth), kiwicha tostada (toasted amaranth), maca, maca tostada, maiz (corn), quinoa, soya (soy), trigo (wheat), and trigo tostada (toasted wheat).

Infrared spectral data was attained using a portable ATR-MIR spectrometer and replicates were taken in triplicate. The portable ATR-MIR spectrometer used (Cary 630, Agilient Technologies Inc., Santa Clara, CA) collected spectra using a single bounce diamond ATR crystal over the characteristic spectral range (4000 – 7000 cm^{-1}). Background readings were taken before gathering the spectra of a new sample. MicroLab FTIR software was used to study the absorbance values present in the spectra collected. In preparation for Gas Chromatography analysis the fat was extracted from all flour samples using pentane for the isolation of fat molecules and their derivitization.

ATR-MIR Spectrometer data was examined using multivariate analysis software (Pirouette version 4.0, Infometrix Inc., Woodville, WA, USA). The spectra were differentiated using Soft Independent Modeling of Class Analogy (SIMCA), which is a supervised method for sample classification that allocates training sets to classes and then a primary components model is created for each class with dissimilar confidence areas (De Maesschalck and Candolfi, 1999). SIMCA was used to characterize the pure flour samples and create a predictive model that was used to either confirm or deny the occurrence of adulteration in the market samples. Adulteration of the marketplace flour samples was suspected if they did not fall within the predicted orbital created by SIMCA. SIMCA's discriminating power was used to determine what functional group absorbencies ATR-IR spectrum provided the ability to make each flour type uniquely different (Kvalheim and Karstang, 1992). An interclass distance greater than three signified that a distinguishable difference in flour types (Kvalheim and Karstang, 1992).

Results & Discussion

Figure 1 illustrates what the typical spectrum of Andean flours would look like when using attenuated total reflectance (ATR) mid-infrared spectroscopy. Characteristic vibrations can be associated with specific functions groups. Therefore, each spectrum shows a characteristic absorbance as wavelengths that are indicative of the water, fat, protein, and starch present in the flour. Although two different types of flour can have similar amount of fat (i.e. soybean and quinoa flours), their spectra would not look identical because that amount of fat can be composed of different fatty acids, which would create a different absorbance pattern on the spectra.

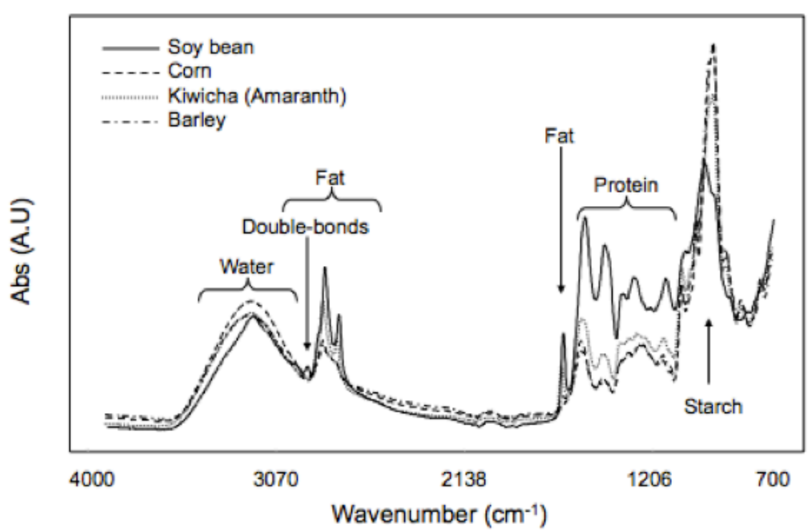

Figure 1. Typical ATR-IR Spectrum of Andean Grain Flours

Both the absorbance data and patterns were inputted into SIMCA to classify the different grains into characteristic groupings that can be seen in Figure 2. The different types of flours were successfully discriminated from one another and placed into their respective orbitals. The orbitals are depicted along three axis and orbitals that reside in closest proximity are the most chemically similar compared to orbitals are located in a farther proximity from one another.

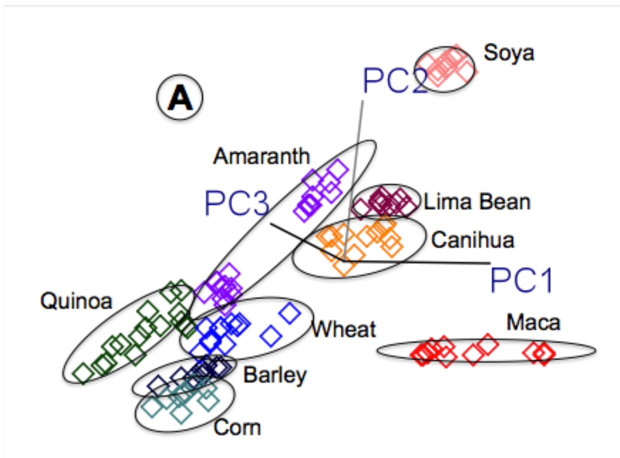

Figure 2. SIMCA Classification of Andean Flours Based on IR spectra of Flour Samples

The interclass distance can be defined as the distance between the gravitometric centers of each orbital created ((Kvalheim and Karstang, 1992). The interclass distances of the various Andean flours tested are exhibited in Table 1showing that most of the flour samples analyzed exhibited an interclass distance greater than a value of three. However, a few of the instances where this was not the case can be observed when comparing toasted lima bean and lima bean flour. Therefore, it is expected that these flours would be harder to statistically separate from one another because they are coming from the same origin. Once the interclass distances were evaluated and considered acceptable the original SIMCA classification model algorithm was used to predict samples acquired from local markets in Lima (Peru).

Table 1. Interclass Distances Between Different Flour Types Using ATR-IR Spectrometer

Canigua	Canigua	Barley[a]	Lima Bean[b]	Amaranth[c]	Toasted Amaranth[c]	Maca	Maca Tostada	Corn[d]	Quinoa	Soy[e]	Wheat[f]	Toasted Wheat[f]	Toasted Barley[a]
Barley[a]	6.2												
Lima Bean[b]	4.3	11.8											
Amaranth[c]	2.5	3.7	4.2										
Toasted Amaranth[c]	6.8	5.7	16.9	3.4									
Maca	18.9	48.1	39.8	24.7	76.5								
Maca Tostada	16.0	45.1	36.8	22.0	77.6	5.9							
Corn[d]	8.5	11.2	14.3	7.8	19.6	41.1	38.5						
Quinoa	6.5	9.0	12.5	6.7	11.7	39.5	36.6	7.7					
Soy[e]	13.3	20.3	17.9	10.3	49.3	73.8	73.8	33.4	26.3				
Wheat[f]	4.6	3.5	8.7	3.5	6.8	36.6	34.3	6.2	5.8	19.1			
Toasted Wheat[f]	5.0	1.9	9.7	3.2	4.5	31.3	28.7	6.1	5.2	18.9	1.1		
Toasted Barley[a]	6.0	4.4	16.4	3.9	30.2	78.6	79.9	13.1	11.2	47.2	4.0	2.1	
Toasted Lima Bean[b]	5.0	16.9	2.8	4.7	49.5	67.5	66.0	18.0	17.2	29.9	10.6	11.4	40.9

Figure 3 represent the market samples whose true composition was tested by our classification model. The market samples were run through a model using the data created from the pure flour samples provided by the Universisad Nacional Agraria. The position of the market sample data points in specific orbitals indicated if adulteration had taken place. For example, if the market sample marketed as soya was truly composed of soya it would fall into the characteristic soya cluster. Further examination of Figure 3 shows that Amaranth fell into the quinoa and lima bean clusters, meaning that it was likely adulterated with those less expensive grains. Additionally, the market sample of quinoa slightly fell into the characteristic wheat cluster. Amaranth and quinoa were the two most expensive grain flours tested and the only two that indicated adulteration.

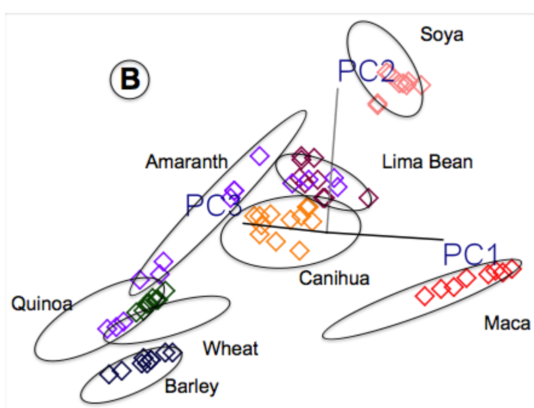

Figure 3. SIMCA Prediction of Andean Flours Based on IR spectra of Flour Samples

Comparing the fatty acid profiles attained from the pure flour samples and the fatty acid profiles attained from market samples provided values that were not significantly different. The most important fatty acids that were being evaluated in the flour samples were palmitic (16:0), stearic (18:0), oleic acid (18:1), and linoleic acid (18:2). There was also very little fatty acid different observed between the toasted and non-toasted samples, except that toasting the samples resulted in elevated levels of stearic acid.

Table 2. Average Percent Area of Major Fatty Acids Present in Various Andean Flour Samples Provided by UNALM

University Provided	C16.0	C18.0	C18.1	C18.2	C18.3	C20.0	C20.1	C22.1	C24.0	C24.1
Canigua	23.5	0	27.0	45.6	3.8	0.0	0.0	0.0	0.0	0.0
Barley[a]	38.1	2.8	26.2	30.3	0.0	0.3	0.7	0.2	0.6	0.6
Lima Bean[b]	14.8	0.8	25.5	48.5	1.0	0.7	0.0	0.0	0.0	3.0
Amaranth[c]	18.4	2.4	27.4	40.5	1.0	1.7	0.0	3.6	0.0	0.1
Toasted Amaranth[c]	17.4	27.4	14.4	29.9	1.8	0.3	0.2	2.2	0.3	0.1
Maca	13.3	0.0	30.2	25.8	2.4	0.2	0.0	0.0	0.0	0.0
Maca Tostada	13.3	0.0	30.2	49.3	2.4	0.2	0.1	0.7	0.0	0.0
Corn[d]	23.9	0.0	30.2	45.6	0.0	0.0	0.0	0.0	0.0	0.0
Quinoa	10.2	0	26.1	51.1	1.1	2.0	0.3	3.2	0.4	0.0
Soy[e]	10.7	3.9	25.0	51.4	2.2	4.6	0.2	0.1	0.1	0.1
Wheat[f]	57.0	16.9	6.4	17.6	0.1	1.2	0.1	0.0	0.3	0.2
Toasted Wheat[f]	17.6	0.2	18.6	57.9	3.8	1.1	0.1	0.0	0.0	0.0
Toasted Barley[a]	34.4	13.5	46.8	5.4	0.0	0.0	0.0	0.0	0.0	0.0
Toasted Lima Bean[b]	35.1	9.4	29.3	20.0	1.4	0.1	0.0	3.4	0.0	0.1

Initially, fat content was hypothesized to be the discriminating factor between the different flours due to their characteristically high fat contents. Figure 4 indicates that the discriminating power was actually found in the protein and starch content of the flour samples as the most important bands describing the discrimination scores were associated with the protein (1400-1650 cm^{-1}) and carbohydrate (1000 – 1200 cm^{-1}) regions. The next steps in this study might be to study and characterize the protein quality in the various flour samples since they are responsible for making each flour class uniquely different.

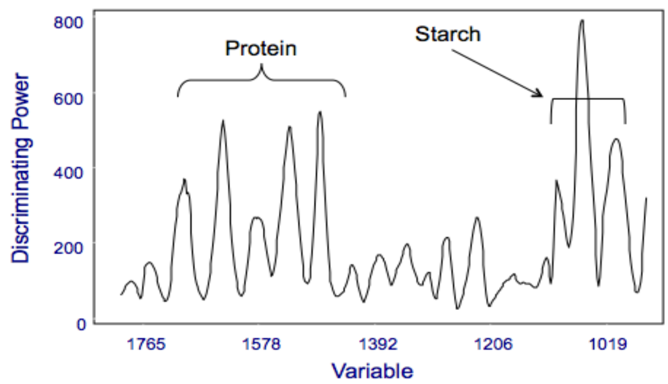

Figure 4. Discriminating Power Based on Infrared Spectra of Grain Flour Samples Using ATR-IR

Conclusion

The Andean flours were successfully separated and distinguished from one another by utilizing FT-IR spectroscopy and chemometrics. Although the fat content in Andean grain flours is exceptionally high, it was not found to be a differentiating factor between the pure and market flour samples. The most important bands describing the discrimination scores were associated with the protein region (1400-1650 cm^{-1}) and carbohydrate region (1000 – 1200 cm^{-1}). Therefore, to continue this study it would be beneficial to compare and analyze the protein composition of these flours. The evaluation of several market flour samples indicated that the adulteration of Andean grains could be occurring, specifically with amaranth and quinoa flours. Amaranth and quinoa were likely adulterated with less expensive grains, such as wheat and lima bean flour. Ultimately, an accurate and high-throughput technique to authenticate Andean grains was demonstrated and can be utilized in-field applications.

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